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Detection of proteins in membrane fractions

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An abbreviated version of this protocol was published in eLIFE in Feb 2021

Membrane-partitioned cell wall synthesis in mycobacteria

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Detailed protocol

Detection of proteins in membrane fractions

This protocol assumes that bacterial lysates were separated in a density gradient, and that membrane fractions were already collected. See Morita *et al*. (2005) and Hayashi *et al*. (2016) for more details.

Materials:

a. 2 gels / 1 gel & 1 place holder
b. Ladder marker
c. Sample Loading buffer: 2X or 4X Reducing (+DTT or β-mercaptoethanol).
d. Running buffer
e. Samples
f. PDVF membrane

Protocol

1.Have the membrane fractions ready. If frozen, thaw them completely on ice.
2.Vortex your tube that contains membrane fractions until you have an homogeneous sample, then:
a) if using 4X sample loading buffer, mix 3 parts sample to 1 part of buffer (24 µL sample + 8 µL sample loading buffer) or
b) if using 2X sample loading buffer, mix 1 part of sample to 1 part of buffer (15 µL sample + 15 µL sample loading buffer).
3. Depending on the proteins of interest (see notes for examples), leave the samples on ice for 30 minutes or boil them for 3-5 min at 95ºC and then chill on ice.
4. Load 12-15 µL of the samples on polyacrylamide gel. The concentration of the gel varies based on the size of the proteins to be visualized.
5. Run the gel at 25 milliampere per gel, approximately for 40 min or as needed.
6. Transfer the separated proteins in the gel to a PVDF membrane, or visualize proteins by in-gel fluorescence directly.
7. If performing an immunoblot, follow the usual protocol performing blocking of the membrane, and incubating with the corresponding primary and secondary antibodies.

Notes:

- Mixture of the membrane fraction sample with sample loading buffer (step #3 of this protocol) can be left on ice or boiled (95ºC/3-5 min) depending on the protocol followed. For example, to visualize the PM-CW marker MptA, samples are incubated on ice. However, after incubating membrane fractions with Bocillin-FL (to label active PBPs, see García-Heredia *et al*. (2021)), samples are boiled for 3 min per labeling protocol.
- In-gel fluorescence imaging takes place when visualizing compatible fluorescent fusion proteins directly on the gel in a compatible imager.
- This protocol was requested from García-Heredia *et al*. (2021).

References

García-Heredia, A., Kado, T., Sein, C. E., Puffal, J., Osman, S. H., Judd, J., . . . Siegrist, M. S. (2021). Membrane-partitioned cell wall synthesis in mycobacteria. *Elife, 10*. doi:10.7554/eLife.60263

Hayashi, J. M., Luo, C. Y., Mayfield, J. A., Hsu, T., Fukuda, T., Walfield, A. L., . . . Morita, Y. S. (2016). Spatially distinct and metabolically active membrane domain in mycobacteria. *Proc Natl Acad Sci U S A, 113*(19), 5400-5405. doi:10.1073/pnas.1525165113

Morita, Y. S., Velasquez, R., Taig, E., Waller, R. F., Patterson, J. H., Tull, D., . . . McConville, M. J. (2005). Compartmentalization of lipid biosynthesis in mycobacteria. *J Biol Chem, 280*(22), 21645-21652. doi:10.1074/jbc.M414181200

Related files

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. García-Heredia, A. , Kado, T. , Sein, C. E., Puffal, J. , Osman, S. H., Judd, J. , Gray, T. A., Morita, Y. S. and Siegrist, M. S.(2021). Detection of proteins in membrane fractions. Bio-protocol Preprint. bio-protocol.org/prep869.
2. García-Heredia, A., Kado, T., Sein, C. E., Puffal, J., Osman, S. H., Judd, J., Gray, T. A., Morita, Y. S. and Siegrist, M. S.(2021). Membrane-partitioned cell wall synthesis in mycobacteria. eLIFE. DOI: 10.7554/eLife.60263